Biomedical Exploitation of Exosomes Delivered in Hydrogels

Subjects: Medicine, Research & Experimental

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Hydrogels are three-dimensional macromolecular polymeric networks composed of hydrophilic polymer chains. Exosomes functioning in the delivery of functional cargos are an active research hotspot. The biological features of exosomes make them suitable as potential therapeutics for the diagnosis and treatment of several diseases.

Keywords: composite hydrogel ; exosome ; biomedical engineering

1. Introduction

Hydrogels are three-dimensional macromolecular polymeric networks composed of hydrophilic polymer chains. They can generally be divided into three categories according to their origin: natural, synthetic, and hybrid. Hydrogels are degradable, with a high affinity for water, and can be fabricated under physiological conditions, resulting in excellent biocompatibility [1]. They can be formed chemically and/or physically upon initiation with crosslinking agents and produced with a certain viscosity and elasticity. The innovation of Wichterle and Lim pioneered a new approach to applying crosslinked hydroxyethyl methacrylate (HEMA) hydrogels as biomaterials in 1960 [2]. In the two decades following this discovery, Lim and Sun demonstrated calcium alginate hydrogels with applications in cell encapsulation [3]. It is not surprising that hydrogels, having mechanical and structural properties similar to those of many tissues and the extracellular matrix (ECM), have attracted great attention, and significant progress has been made in designing, synthesizing, and using these materials for many biological and biomedical applications [4].

Exosomes are small, single-membrane, secreted extracellular vesicles (EVs), enriched in certain proteins, nucleic acids, and lipids. Budding at both the plasma and endosomal membranes of all the mammalian cell types studied to date, they are produced to remodel the ECM and deliver signals and functional macromolecules to adjacent cells. Numerous surface molecules on exosomes enable them to be internalized via endocytosis by recipient cells, playing an important role in regulating cell–cell communication [5]. Therefore, the study of exosomes in the pathology of various diseases is an active area of research, and the exploration of therapeutic exosomes as delivery vesicles has offered new insights for clinical applications in recent years. However, the stability and retention of exosomes released in vivo are major hurdles, as they are rapidly cleared by the innate immune system or accumulate in the liver, spleen, and lungs via the blood circulation [6].

The biological features of exosomes make them suitable as potential therapeutics for the diagnosis and treatment of several diseases. There are generally three approaches to obtaining exosomes with therapeutic and diagnostic potential. (1) Naturally derived exosomes (e.g., MSC-Exos) have been verified to be therapeutic by themselves [7]. (2) Engineering exosomes by transferring molecules such as microRNAs has achieved targeted applications [8]. (3) Exosome mimetics have been exploited as promising biomaterials [9][10].

2. Tissue Repair

Of the many classes of biomaterials that have been used in tissue repair, hydrogels have been regarded as one of the most prominent and versatile for supporting most cellular behaviors and nutrient transport. Protected by them, cellular secretions can maintain their biological activity and undergo controlled release in pathological environments (**Table 1**).

Table 1. Advances in tissue regeneration via the hydrogel encapsulation of EVs.

Composite Hydrogel Type	Exosome Source	Release Kinetics	Therapeutic Application	Reference
GelMA/nanoclay hydrogel	hUCMSCs	90% in a month	Cartilage regeneration	[11]
HA hydrogel	ECs	80% in a week	Fracture repair	[8]

Composite Hydrogel Type	Exosome Source	Release Kinetics	Therapeutic Application	Reference
GMOCS hydrogel	BMSCs	80% in 2 weeks	Repair of growth plate injuries	[12]
PEO–PPO–PEO hydrogel	PRP	80% in 20 days	Subtalar osteoarthritis	[13]
Pluronic F-127 hydrogel	Melanoma cells	Release peaked at 24 h	Chronic wound repair	[14]
HA@MnO hydrogel	M2	Over 80% in 21 days	Repair of chronic diabetic wounds	[15]
Methylcellulose–chitosan hydrogel	PMSCs	Not mentioned	Severe wound healing	[16]
HA hydrogel	iPS-CPCs and iPS-MSCs	Lasting over 2 weeks	Cardiac remodeling after MI	[17]
AT-EHBPE/HA-SH/CP05 hydrogel	hUCMSCs	Not mentioned	MI and reperfusion injury	[18]
Gelatin–laponite nanocomposite hydrogel	hADSCs	Not mentioned	Repair of peri-infarct myocardium	[19]
PDNP–PELA hydrogel	ADSCs	92.5 ± 5.7% in 2 weeks	Erectile dysfunction treatment	[20]
Peptide-modified HA hydrogel	hPAMMSCs	80% in a week	Recovery from spinal cord injury	[21]
Chitosan hydrogel	DPSCs	80% in a week	Periodontitis	[7]
Fibrin hydrogel	Rat BMSCs	Left over 2 weeks	Tendon regeneration	[22]

hUCMSC (human umbilical cord mesenchymal stem cell); EC (endothelial cell); BMSC (bone marrow mesenchymal stem cell); OCS (chondroitin sulfate); GM (gelatin macryloyl); PRP (platelet-rich plasma); M2 (M2 macrophage); PMSC (placental mesenchymal stem cell); iPS (induced pluripotent stem cell); CPC (cardiac progenitor cell); MI (myocardial infarction); AT (aniline tetramer); EHBPE (epoxy macromer); HA-SH (thiolated hyaluronic acid); hADSC (human adipose-derived stem cell); PDNP (polydopamine nanoparticle); PELA (poly(ethylene glycol)poly(ε-caprolactone-co-lactide)); hPAMMSC (human placenta amniotic membrane mesenchymal stem cell); DPSC (dental pulp stem cell).

2.1. Bone and Cartilage Defects

Overwhelming evidence shows that the exogenous transport of miRNAs by exosomes can regulate osteogenic and angiogenic differentiation. An example of this is a study carried out by Mi et al., who created a cocktail therapy by transferring miR-26a-5p into endothelial cell-derived exosomes (EC-Exos) in an HA hydrogel. The EC-Exos$^{miR-26a-5p}$ promoted osteogenic and osteoclast differentiation in mice with femoral fractures [8]. In another study, Hu et al. found that human umbilical cord MSC-derived small EVs (hUCMSC-sEVs) activated the PTEN/AKT signaling pathway by transferring miR-23a-3p when investigating the role and mechanism of cartilage regeneration [11]. Compared to increasing the specific miRNA in the target cells, the inhibition of miR-29a was verified to stimulate endogenous BMP/Smad signaling, which triggers subsequent osteogenic differentiation [9]. Therefore, the overexpression of miRNA can be an attractive method for improving the therapeutic effects. For example, miR-375 could be enriched in human adipose MSC (hASC)-derived exosomes by overexpressing the miRNA cargo in the parent cells [23].

Extensive research has shown that the essential properties of a bone and cartilage engineering scaffold are mechanical strength and a porous structure, to support the attachment and infiltration of osteogenic cells [24]. Hu et al. recently utilized an injectable and UV-crosslinked gelatin methacrylate (GelMA) to fabricate with nanoclay and achieved the sustained release of small EVs with the degradation of the hydrogel. The addition of laponite nanoclay significantly enhanced its ultimate strength for local administration in cartilage defects [11]. In addition to additives, 3D technology can also be applied to customize the shapes and sizes of porous scaffolds in accordance with bone defects. Fan et al. encapsulated umbilical MSC-derived exosomes (UMSC-Exos) in an HA hydrogel and combined them with 3D-printed nanohydroxyapatite/poly-ε-caprolactone (nHP) scaffolds [25]. Taken together, hydrogels can regulate extracellular matrix (ECM) formation, which provides a three-dimensional (3D) culture system for exosome secretion [26][27].

2.2. Wound Repair

As a complicated biological process, wound healing consists of inflammation, proliferation, and remodeling [28]. The conventional treatment of chronic wounds includes regular wound debridement for stimulating skin regeneration and the

protection of the wound using a specific dressing [29]. Recent interventions inspired by cell therapy approaches involve exosomes derived from MSCs, plasma, and cancer cells, while stem cell-derived exosomes are being developed for tissue recovery [14][30][31]. In a diabetes-impaired wound model, a wound dressing biomaterial was applied by combining antioxidant polyurethane (PUAO) for attenuating oxidative stress and adipose-derived stem cell (ADSC) exosomes for tissue remodeling [31]. Similarly, immobilizing ADSC-derived exosomes in a composite hydrogel that includes poly-ε-L-lysine (EPL), a natural cationic polypeptide from Streptomyces albulus, can help to realize antibacterial activity and adhesive ability [32]. Another study explored the feasibility of a composite hydrogel formed from silk fibroin (SF) and silk sericin (SS) due to the excellent mechanical properties of SF, and the cell-adhesion and biocompatibility properties of SS. After encapsulating and delivering UMSC-Exos, SF–SS hydrogels promoted wound healing and angiogenesis [33]. Additionally, the delivery of platelet-rich plasma exosomes in a composite chitosan–silk hydrogel sponge was found to upregulate collagen synthesis and deposition, as well as angiogenesis, at the wound site in diabetic rat models [30]. In addition, exosomes were enriched in miR-21, miR-23a, miR-125b, and miR-14, which can be blocked to reduce scar formation when they are laden in hydrogels [34]. Chitosan hydrogels functionalized with exosomes from synovium MSCs transduced to overexpress miR-126 promoted healing and angiogenesis in skin wounds [35].

2.3. Cardiovascular Diseases

Ischemic myocardial infarction (MI) results from the severe blockage of blood arteries, which, in turn, interrupts nutrient supply. However, clinical treatments may lead to further myocardial ischemia/reperfusion injury [36]. New findings have triggered studies investigating the potential of utilizing MSC-derived EVs after MI to promote angiogenesis and restore cardiac function [19][37][38][39]. For example, Zou at al. elaborated an exo-anchoring conductive hydrogel enabling electrical conduction within the myocardial fibrotic area and promoting the synchronous contraction of the myocardium. In this study, an aniline tetramer (AT) was employed as a crosslinker, and the researchers endowed it with electroconductibility. The CP05 peptide was applied for its capability of binding to CD63 on the exosomal surface, to anchor and capture exosomes from human UC-MSCs [18]. Based on the intended application, hydrogels can be synthesized with different preparations. A notable application is to encapsulate EVs from induced pluripotent stem cells in a hydrogel patch and apply them directly onto the rat myocardium. The hydrogel patch enabled sustainable release, which protected the acutely injured heart against pathological hypertrophy [26].

2.4. Spinal Cord Injury

Spinal cord injury (SCI) is among the most fatal diseases of the central nervous system, resulting in a temporary or permanent loss of sensation, movement, strength, and body functions [40]. To overcome the low cell survival resulting from the inhibitory environment at the lesion site, the local injection of exosomes protected by hydrogels is a promising therapeutic strategy. Li et al. improved the affinity of HA hydrogels and MSC-derived exosomes by a laminin modification, and successfully promoted spinal cord regeneration and the recovery of hindlimb motor function in vivo [21]. Surprisingly, plant (e.g., ginseng)-derived exosomes that can stimulate the neural differentiation of BMSCs have been demonstrated, and can be loaded in GelMA to fit the irregular shapes of injury defects [41]. The promotion of angiogenesis is beneficial for the regeneration of neuronal networks after SCI. Inspired by this, Luo et al. utilized a hybrid hydrogel system comprising GelMA, HA-NB, and a photoinitiator (LAP) to immobile exosomes from M2 macrophages. The hydrogel-mediated release system protected the exosomes from severe oxidative stress and inflammation [32].

2.5. Other Diseases

In addition to the aforementioned applications, exosomes have also played important roles in periodontal, endometrial, and corneal repairs. In the context of periodontitis, the incorporation of dental pulp stem cell-derived exosomes and chitosan hydrogels repolarized macrophages and accelerated periodontal regeneration [7]. The dynamic coordination of adipose stem cell-derived exosomes and PEG hydrogels via Ag^{+}–S resulted in outstanding injectable, self-healing, and antibacterial properties for endometrial and fertility restoration [15]. To effectively promote the repair of corneal damage, exosomes derived from MSCs were loaded in thermosensitive chitosan-based hydrogels [42].

3. Immune Regulation

Commonly, the adaptive immune response is regulated by antigen-presenting cells (APCs), such as dendritic cells (DCs), B cells, and macrophages, directly interacting with T cells and natural killer (NK) cells through cell-surface proteins [43]. Exosomes produced by APCs play an important role in the regulation of immunity, mediating immune stimulation or suppression, and driving inflammatory, autoimmune, and infectious disease pathology [44]. Inspired by dendritic cell-derived exosomes (DEXs), which improve cardiac function by activating $CD4^{+}$ T cells in the spleen and lymph nodes [45], Zhang et al. encapsulated DEXs in a simple alginate hydrogel and injected the DEX-Gel into the MI model. The DEXs

significantly upregulated the infiltration of Treg cells and M2 macrophages, which resulted in better wound remodeling, and preserved systolic function after MI. Furthermore, the combined application of the hydrogel provides physical support to the infarcted area [46].

MSCs confer regenerative effects in different tissue injuries, while in some cases, MSCs have been confirmed to secrete immunosuppressive cytokines and other factors, resulting in anti-inflammatory effects from stem cells [47]. Notably, the analysis of MSC-derived EVs revealed that they also have immunosuppressive therapeutic effects [48]. To harness EVs' immunosuppressive properties, Fuhrmann et al. innovatively incorporated enzyme-loaded vesicles from MSCs into PVA hydrogels and applied this bioactive material for enzyme prodrug therapy. Once vesicles are released into the desired site, the injected nontoxic prodrugs are converted to anti-inflammatory drugs by enzymes [49]. The polarization of M2 macrophages, which can inhibit inflammation and induce tissue regeneration, has recently drawn great attention [7][8][50]. A classic cue is osteoimmunology, in which exosomes overexpressing miR-181 from human bone marrow-derived MSCs (hBM-MSCs) combined with a hydrogel were verified to significantly enhance osseointegration [50].

Tumor-derived EVs have been revealed to suppress tumor-specific and non-specific immune responses [44]. Metastatic melanoma releases a high level of exosomes carrying PD-L1 on their surfaces, which help in the evasion of immune surveillance. Based on how tumor cells suppress the immune system, Su et al. isolated exosomes from melanoma cells overexpressing PD-L1 to decrease T cell proliferation in a wound-healing model. The application of the thermoresponsive Pluronic F-127 hydrogel ensured that exosomes were released in a sustained manner [14].

4. Pathogenesis Study

Along with mediating physiological intercellular communication, exosomes also spread pathogenetic cargoes in diseases. Identifying the proteins and RNAs of exosomes can provide therapeutic targets. However, exosomal behavior can be dictated by the environment [4]. Therefore, hydrogels providing certain mechanical, structural, and compositional cues in the extracellular microenvironment are adopted as a novel strategy to recapitulate numerous physiologically relevant cell behaviors [51].

Tumor-derived exosomes can assist tumor growth and promote metastasis. To demonstrate the role of exosomes in ECM stiffness-triggered breast cancer invasiveness, Patwardhan et al. fabricated stiffness-tunable polyacrylamide (PA) gels as ECM mimics. Interestingly, stiff ECM cultures fostered exosome secretion by a series of changes in cell morphology, adhesion, and protrusion dynamics, which resulted in the invasion of breast cancer cells [52]. Aberrant cell behaviors can be induced by in vitro 2D culture, and the heterogeneity of exosomal behaviors also depends on the culture conditions [53]. Therefore, Millan et al. created 3D-engineered microtissues using the polysaccharides alginate and chitosan for the study of prostate cancer-derived EVs. Proteomics and RNA sequencing comparing 2D- and 3D-cultured cells revealed significantly differential expression of EV biomarkers. Some proteins known to be drivers of prostate cancer progression that were not detectable in the 2D conditions were enriched in the 3D cultures [54].

Exosomes from different cells such as endothelial cells and smooth muscle cells can contribute to atherosclerosis and cardiovascular disease when circulating in the blood [55][56]. In atherosclerosis-prone areas, EVs from smooth muscle cells (SMCs) and valvular interstitial cells (VICs) can cause a phospholipidic imbalance and, consequently, vascular and valvular calcification. Three-dimensional collagen hydrogels were utilized to produce a cardiovascular calcification model with which to observe the aggregation and microcalcification at the EV level [57]. Moreover, lesion macrophages can deliver exosomes that regulate vascular SMCs during the progression of atherosclerosis. In a study investigating the potential role of exosomes from nicotine-treated macrophages, Zhu et al. incorporated the above exosomes with chitosan hydrogels to stimulate release at the abdominal aorta [58].

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